Materials and methods: This exploratory analysis was performed within a prospective phase II study to determine the clinical outcome and the incidence of EGFR mutations in male smokers with squamous cell carcinoma, who were treated with EGFR tyrosine kinase inhibitors. We analyzed the incidence of EGFR mutations in NSCLC specimens from 69 Korean patients who were treated with gefitinib or erlotinib in a prospective study. For a subset of 20 male patients with squamous cell carcinoma and a history of smoking, pretreatment tumor tissue samples were obtained and analyzed for EGFR mutations (exons 18 to 21).

Results: EGFR mutations were found in 3 (15%) out of 20 patients, including in-frame deletions within exon 19 (n=2) and L858R missence mutation in exon 21 (n=1). The 3 patients with EGFR mutations responded to EGFR inhibitor therapy, whereas only one of remaining 17 patients with wild-type EGFR achieved clinical response. Trends toward longer progression-free survival (5.8 vs. 2.4 months; P=0.07) and overall survival (9.6 vs. 7.2 months; P=0.76) were noted in patients with EGFR mutations compared to those with wild-type EGFR, respectively.

Conclusions: Although male smokers with squamous cell carcinoma have not been considered ideal candidates for treatment with EGFR tyrosine kinase inhibitors, significant incidence of EGFR mutations was observed.

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A decline in circulating HER2 DNA predicts treatment response and survival in breast cancer patients treated with trastuzumab

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Some, but not all breast cancer patients, who over express HER2 (human epidermal growth factor receptor 2), respond to the HER2 inhibitor trastuzumab. It is the purpose of this study to identify patients responding to trastuzumab treatment by a novel technique measuring the amplification of the HER2 gene in DNA circulating in the plasma of patients with metastatic breast cancer. An already established method determines the release of the extracellular domain (ECD) of HER2 into the circulation, and it is the aim to compare the two methods.

DNA was isolated from plasma collected just prior to the start of trastuzumab treatment from 28 patients with metastatic breast cancer who all contained an amplified HER2 gene in their primary tumour. From 22 patients an additional blood sample was also collected three weeks after the start of trastuzumab treatment. HER2 gene amplification was measured with real time PCR and expressed relative to the un-amplified gastrin gene. The cut off value (ratio of 1.14) was calculated based on analysis of plasma from 20 control subjects without breast cancer (mean + 2 SD). HER2 ECD was measured with the HER2/neu kit (Bayer), and the cut of value given by the manufacturer (15 ng/ml) was used.

Prior to treatment, HER2 DNA was increased in 52% and HER2 ECD was increased in 57% of the patients, but in neither case did this correlate to treatment response or overall survival. In 9 of 22 patients a reduction in the amount of the amplified HER2 gene in the circulating DNA was observed following trastuzumab treatment (more than 14% (2 SD) was considered a reduction). This reduction correlated to treatment response (P=0.02, log rank test), as well as to an improved overall survival (P=0.05). No correlation between clinical data and the kinetics of HER2 ECD was observed.

In conclusion we demonstrate that the dynamics of circulating HER2 DNA following trastuzumab treatment predict treatment response in patients with metastatic breast cancer. In contrast to previous studies we did not observe a correlation between HER2 ECD and response to trastuzumab.

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Resistance to cetuximab - implication of PTEN expression in the cellular sensitivity to cetuximab (Erbitux®) of Head and Neck Squamous Cell Carcinoma (HNSCC)

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Targeted therapies using monoclonal antibodies and tyrosine kinase inhibitors are very efficient for treatment of different cancers and theoretically less toxic than usual therapies. Nevertheless, a significant number of resistance cases to targeted therapies has been reported. In case of cetuximab (Erbitux®), a chimeric human/murine monoclonal antibody targeting Epidermal growth factor receptor (EGFR or Human Epidermal Receptor 1, HER1), such acquired resistance has been reported in colorectal treatment and explained by an overproduction of vascular endothelium growth factor (VEGF). Because of its control on the VEGF expression in tumoral cells, the Pi3K/AKT signalling pathway would be the true resistance mechanism and more specially, the protein PTEN (a tumor suppressor gene) and AKT phosphorylation.

The aim of our study is to precise the roles of PTEN and phospho-AKT proteins in a quite new indication of cetuximab: Head and Neck Squamous Cell Carcinoma (HNSCC) by using small interfering RNA. For this purpose, Cal 27 (human HNSCC cell line) choosen as a model of sensitive cell line to cetuximab, was transfected by PTEN-siRNA and then treated by cetuximab during 48 hours in the period of inhibition of PTEN expression. Cell cycle analysis was performed by flow cytometry after 24 hours of exposition to cetuximab. Proteins extraction and MTT assays were done after 48 hours of exposition. Western blot and Bioplex proteins array were used to check PTEN and p-AKT expression and to evaluate activation level of signalling pathways. Our results showed a significant increase of cell proliferation and metabolic activity, correlated with a significant increase of AKT phosphorylation in PTEN-SiRNA transfected cells versus non transfected cells after cetuximab treatment.

In conclusion, we demonstrated that the loss of expression of tumor suppressor gene PTEN in human HNSCC cell line, creates a resistance to cetuximab. This loss of PTEN expression would have consequences on VEGF expression.

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Preclinical evaluation of dasatinib in melanoma cell lines

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Introduction: Newer targeted therapies, alone and/or in combination with chemotherapy, offer new hope of improving prognosis for malignant melanoma. In this study we evaluated the potential benefits of dasatinib as a targeted therapy for melanoma.

Methods: Dasatinib was tested alone and in combination with temozolomide in a panel of melanoma cell lines, using the acid phosphatase proliferation assay. The effects of dasatinib on invasion, migration, apoptosis and cell cycle arrest were assessed in the melanoma cells. Expression of Src kinase and EphA2, and the effect of dasatinib treatment on expression and activation of Src and EphA2 were measured by immunoblotting.

Results: Four of the six melanoma cell lines were responsive to dasatinib. Lox-IMVI had an IC50 of 35.4 nM (± 8.8 nM). HT144, Malme-3M and M14 also display sensitivity with a maximum growth inhibition of 40 %, 30 % and 15 %, respectively, achieved in these cell lines with 1 μM dasatinib. When combined with temozolomide in both HT144 and Malme-3M, dasatinib enhanced response to temozolomide. In Lox-IMVI, CI values (CI value at ED50 = 0.88) revealed the combination of dasatinib and temozolomide was slightly synergistic. Dasatinib significantly decreased invasion of both HT144 and M14 cells (in M14 dasatinib (25 nM) reduced invasion by 65 %; p = 0.005). Dasatinib also significantly decreased the level of migration in HT144 and M14 in a dose dependant manner (in M14 dasatinib (25 nM) reduced migration by 83 %; p = 0.004). Dasatinib (200nM) induced apoptosis after 72 hours in LOX-IMVI (12 %) and Malme-3M (20 %). Cell cycle analysis of dasatinib treatment in the melanoma cells indicated that dasatinib increased G1 arrest in HT144 (17 %) and Lox-IMVI (23 %). Src kinase and low levels of phosphorylated Src kinase were detected in all cell lines tested. The level of Src kinase phosphorylation decreased in HT144, Lox-IMVI and Malme-3M when treated with dasatinib, but the level of phosphorylation was increased in Sk-Mel-28 cells treated with dasatinib. Finally EPHA2 expression was higher in the dasatinib sensitive cell lines, so EPHA2 expression can predict response to dasatinib in our panel of cell lines.

Conclusions: Our results show that dasatinib has anti-proliferative, pro-apoptotic and anti-invasive effects in dasatinib-sensitive melanoma cells. Therefore, combining dasatinib with temozolomide, may improve response to treatment in these tumours.